Recommendations for the Generation and Submission of Genomic Data

Concept Paper

Draft — Not for Implementation

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Recommendations for the Submission and Review of Genomic Data — Concept Paper

INTRODUCTION

The discussion in this concept paper expands on the recommendations made in FDA's guidance for industry on *Pharmacogenomic Data Submissions*, issued in March 2005. Based on its experience with voluntary genomic data submissions as well as with its review of numerous protocols and data submitted under investigational new drug (IND) applications, new drug applications (NDAs), and biologics license applications (BLAs) during the last 2 years, FDA believes that guidence will benefit sponsors considering the submission of either voluntary genomic data submissions or marketing submissions containing genomics data. FDA plans to develop such guidance based on the concepts in this paper and input from a public meeting. As technology changes and more experience is gained, these concepts also may change.

1. GENE EXPRESSION DATA FROM MICROARRAYS

The following methodological issues should be considered when submitting gene expression data from microarrays.

1.1. RNA Isolation, Handling and Characterization

The most critical step in performing RNA-based experiments such as microarray gene expression experiments is the isolation of high quality, intact RNA. To achieve this goal and preserve sample integrity throughout the course of the experiment, some steps before and after RNA purification need to be carefully planned to ensure high quality during isolation and confirm high quality before use in a downstream application. A secondary goal is maximizing the yield of RNA. In addition, storage and shipping conditions of samples can influence the stability of RNA. Thus, it is very important to store the RNA under the best conditions to preserve the integrity of the sample. Finally, we recommend that standard operating procedures (SOPs) be established to ensure reproducibility of the RNA isolation method and assurance of RNA quality (e.g., see http://www.fda.gov/nctr/science/centers/toxicoinformatics/maqc/docs/MAQC_Sample_Processing_Overview_SOP.pdf). The following recommendations will help achieve these goals.

Pre-RNA isolation considerations: RNA is sensitive to degradation by RNase, which is ubiquitously present in living organisms. Thus, sample-handling issues need to be addressed before embarking on RNA isolation from samples, and we recommend that work areas and equipment be dedicated specifically for RNA isolation and other RNA-related work.

RNase-free reagents and disposables/glassware: It is imperative to use RNase free reagents and glassware for RNA isolation. Commercially available RNA isolation kits often provide these.

RNA Stabilizer(s): We recommend that you assess the need for adding RNA stabilizing agents to samples/reagents and that an appropriate RNA stabilizer be identified and tested in a pilot experiment.

Batch size: We recommend that the maximal batch size for sample preparation be determined to help identify and limit the time taken for the entire RNA isolation process. Establishing an upper limit for batch size will reduce problems encountered in the scaling-up process, since long processing times can jeopardize RNA integrity.

Sample collection, storage and shipping conditions: The size and thickness of a sample and acquisition of area sampled are critical. A thick slice of tissue may not preserve uniformly or quickly enough to protect RNA quality in the interior of the sample piece. A sample of a tissue or organ representative of the entire tissue/organ is recommended if the entire tissue/organ is not used. Speed of dissection and preservation of tissue are also critical, especially for RNA quality. We also recommend that tissues or cells be quickly frozen in liquid nitrogen and stored at -80^0 C to prevent RNA degradation. Tissues or cells, to which RNA stabilizing agents such as RNA later are added in manufacturer-recommended amounts or proportions, may be stored long term at -20^0 C or -80^0 C. Whole blood should be stored and shipped according to the collection tube manufacturer's specifications. Isolated RNA must be shipped on dry ice to ensure that the RNA sample does not deteriorate during shipping.

Genomic DNA contamination: Selection of a method of RNA isolation that prevents contamination of the isolated RNA with genomic DNA is of great importance, since genomic DNA could negatively affect downstream applications. We recommend you experiment to ensure the lack of a signal from genomic DNA for a given preparation method.

RNA isolation from tissues or cells: Treatment of samples prior to RNA isolation and careful handling are necessary to preserve RNA. This is the first step to getting good quality RNA. RNA can be successfully isolated from fresh tissue and cell culture samples if the sample is immediately homogenized in the presence of stabilizing detergents and chaotropic reagents. Often, an RNA stabilizer that is compatible with RNA isolation procedures is added to the isolated tissues or cells before storing the samples. Alternatively, tissues or cells can be quickly frozen in liquid nitrogen and stored at -80^0 C to prevent RNA degradation. This is particularly useful when the tissues/cells are difficult to homogenize. The frozen material can then be ground and processed with greater ease. Another possibility is to homogenize the tissue/cells in the presence of a strong denaturant that renders RNase inactive and to then freeze the homogenate —however, this may not be universally applicable to all of the available homogenizing solutions. In those cases, we recommend that the manufacturer's specifications be followed. During the actual isolation procedure, it is essential to use RNase-free reagents, equipment or materials, and work spaces. Several RNA isolation kits are commercially available.

RNA isolation from blood: RNA can be isolated from whole blood or from peripheral blood mononuclear cells (PBMCs). Most studies conducted so far have used the PBMCs since they are the most transcriptionally active cells in blood.[1] This fraction primarily consists of lymphocytes and monocytes.

RNA isolation from whole blood: Others prefer to isolate RNA from whole blood since the blood sample with the RNA stabilizer can be stored for a long time without compromising RNA quality under manufacturer-suggested conditions. By using this option, the expression profiles of all the genes would be likely to be conserved. However, there is a disadvantage to RNA isolation from whole blood. Even though reticulocytes (immature red blood cells (RBCs)) represent only 0.5-2% of the RBCs, their RNA, (of which globin mRNA is the major RNA) can contribute up to 70% of the mass of mRNA in total RNA from whole blood. In microarray gene expression experiments, the overabundance of globin mRNA causes non-detection of some transcripts that are less abundant or are low-expressors.[2] Protocols for reducing globin mRNA from whole blood are available.[3]

The quality of the microarray data can be improved by removing reticulocytes, although this often requires that blood be processed at the site of blood draw. Any manipulation of the blood sample may cause a change in the gene expression profiles of some transcripts.[4] No matter what overall protocol is used, we recommend a stability study simulating the conditions of the preclinical or clinical sample collection to be employed, which assesses the impact of key preanalytical variables on the chosen method (time to processing, temperature of storage, etc.).

RNA isolation from PBMCs: Commonly used methods include the Ficoll-Hypaque method and a protocol using cell preparation tubes with sodium citrate. RNA isolation from PBMCs is the preferred method for many, since the RNA is free from globin mRNA and generally gives better results on microarrays. However, it has been shown that time delays and temperature changes can affect gene expression profiles of several genes[5, 6] and that it is critical to isolate the PBMCs

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[1] An Analysis of Blood Processing Methods to Prepare Samples for GeneChip Expression Profiling- Technical Note from Affymetrix. (http://www.affymetrix.com/support/technical/technotes/blood_technote.pdf)

[2] Fan H. (2005) The transcriptome in blood: challenges and solutions for robust expression profiling. *Current Molecular Medicine* **5**, 3-10.

[3] Debey S. et al., (2006) A highly standardized, robust, and cost-effective method for genome-wide transcriptome analysis of peripheral blood applicable to large-scale clinical trials. *Genomics* **87**, 653-664.

[4] For a very recent overview of the multiple approaches available for generating profiles from PBMC or whole blood see Burczynski M.E. and Dorner A.J. (2006) Transcriptional profiling of peripheral blood cells in clinical pharmacogenomic studies. *Pharmacogenomics* **7**, 187-202.

[5] Baechler E.C. (2004) Expression levels for many genes in human peripheral blood cells are highly sensitive to ex vivo incubation. *Genes and Immunity* **5**, 347-353.

[6] Debey S. (2004) Comparison of different isolation techniques prior gene expression profiling of blood derived cells: impact on physiological responses, on overall expression and the role of different cell types. *The Pharmacologics Journal* **4**, 193-207.

within hours of blood collection, in the absence of any material/storage condition that might stabilize the expression profiles of the cells.

RNA QC. The two preferred methods for quality control (QC) of RNA samples are agarose gel electrophoresis or RNA LabChip analysis using the Agilent 2100 Bioanalyzer. Spectrophotometric analysis using the ratio of absorbance at 260nm/280nm can also be used as a measure of RNA quality.[7] Considerations include the following:

- Ratio of absorbance at 260nm and 280 nm (A_{260}/A_{280}) can be used to assess RNA purity and is typically recommended to be greater than 1.8.[8]

- Generally, 1% agarose gel is used, and clearly visible 18S and 28S RNA bands are taken as measure of RNA integrity. Ideally, the intensity of the 28S band should be twice the intensity of the 18S band. Degraded RNA will have a smeared appearance and lack two clear bands.

- With the Agilent Bioanalyzer, the profile generated should have two major peaks corresponding to 18S and 28S rRNA. The ratio of 28S/18S should ideally be 2 (at least greater than 1.4) and the sum of the two rRNA peak areas should account for more than 30% of all RNA (7). A 1 KB RNA fragment should always be spiked in every sample as a marker. In degraded samples, the baseline should be increased between the marker and the rRNA peaks. Also, the 28S rRNA band should shrink, and a broad band may appear close to the spiked marker. The RNA integrity number (RIN) generated by the RIN software tool should be used to assess the integrity of total RNA samples. In addition, a RIN can provide a cut-off threshold for poor quality samples (e.g., RIN $\leq$ 6), while 28S:18S ratio does not.[9]

1.2. Labeling Reactions

In genomic submissions, it is important that sponsors use a labeling system that has been documented to perform well on a given manufacturer's array. It is critical that the sponsor begin the labeling process with high-quality RNA-free of contaminants that might affect the labeling efficiency or introduce labeling bias, as compromised RNA quality will affect subsequent steps of sample processing and ultimately lead to poorer quality microarray data. We recommend that the use of accepted quality measures (RIN or 18/28S ratios) be included in this report and that RNA samples prepared for labeling be of comparable quality.

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[7] http://arrayconsortium.tgen.org/np2/public/qualitycontrol/jsp.

[8] Dumur, C.I. et al., (2004) Evaluation of Quality-control criteria for Microarray Gene Expression Analysis. *Clinical Chemistry* **50**(11), 1994-2002.

[9] Imbeaud S, Graudens E, Boulanger V, Barlet X, Zaborski P, Eveno E, Mueller O, Schroeder A. and Auffray C. (2005) Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. *Nucleic Acids Research* **33**(6), e56.

Use consistent methods of target labeling throughout the particular study or studies that will be analyzed as a group since dissimilar microarray data could be obtained when kits from different manufacturers or different types of labeling kits are used. If there is any change in a critical component in the labeling kit (kit manufacturer, key enzyme or reagent), we recommend that it be tested to demonstrate comparability of the data generated prior to being used with samples analyzed as an arm of a study.

The use of standard operating procedures (SOPs) is encouraged, and we recommend that operators be fully trained on all protocols prior to processing of samples for the study. Equipment should be on an appropriate maintenance schedule and the laboratory environment maintained in accordance with the manufacturer's recommendations.

The development of QC or intermediate labeling steps is highly recommended. If any intermediate QC step indicates a problem and the RNA is of reasonable quality, the labeling process can be repeated to produce higher quality input material for hybridization to the microarray chip. In addition, it is recommended that reagents be stored under appropriate conditions. Use of controls and reference standards are recommended to verify consistent performance throughout the labeling procedure.

1.3. RNA labeling situations to be *avoided*

The following labeling situations should be avoided.

- Starting amplification and labeling reaction with poor quality RNA
- Using expired reagents
- Using reagents stored at less than optimal conditions
- Changing the brand of RNA labeling kit in the middle of a study (this could lead to dissimilar microarray data being generated that should ***not*** be analyzed as a group)

1.4. Proficiency Testing to Avoid Procedural Failures

High-quality data are the foundation for deriving reliable biological conclusions from a microarray gene expression study. However, large differences in data quality have been observed in published data sets when the same platform was used by different laboratories.[10, 11] In many cases, poor quality of microarray data was due not to the inherent quality problems of a platform but to the lack of proficiency of the laboratory that generated the data. Such a *procedural failure* in a laboratory is much more serious than randomly failed hybridizations that lead to outlying arrays. This is because the laboratory may not recognize that it has a procedural failure problem.

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[10] Shi L, Tong W, Goodsaid F, Frueh FW, Fang H, Han T, Fuscoe JC and Casciano DA (2004) QA/QC: challenges and pitfalls facing the microarray community and regulatory agencies. *Expert Rev Mol Diagn* **4**:761-77.

[11] Shi L, Tong W, Fang H, Scherf U, Han J, Puri RK, Frueh FW, Goodsaid FM, Guo L, Su Z, Han T, Fuscoe JC, Xu ZA, Patterson TA, Hong H, Xie Q, Perkins RG, Chen JJ and Casciano DA (2005) Cross-platform comparability of microarray technology: intra-platform consistency and appropriate data analysis procedures are essential. *BMC Bioinformatics* **6 Suppl 2**:S12.

The Agency recommends that sponsors provide data that will enable FDA reviewers to objectively evaluate the competency of the laboratory that generated the data in a genomic submission. Many studies report quality control metrics or use standards to provide internal assessments of microarray data. This information is essential for confirming the technical aptitude of an assay within an individual study. An assessment of the overall competence of a facility can be performed through inter-laboratory comparisons, such as proficiency testing. Laboratory proficiency can be achieved through a number of approaches.

- RNA sources

Two FDA-led initiatives have developed and characterized reference RNA samples for proficiency testing. Mixed tissue pools of rat RNA samples have been designed with known differences in tissue-selective genes[12] and have been used in the first proficiency testing program for microarray laboratories.[13] In addition, the MicroArray Quality Control (MAQC) Project[14] developed two human reference materials and extensively tested them on multiple gene expression platforms. Data from both initiatives have been deposited in public databases, and the RNA samples used in the MAQC project are now commercially available. Thus, individual laboratories can repeat the MAQC experiment in their own facility using the identical RNA samples and the resulting expression data can be compared to those generated by the MAQC project.

- Proposed experimental design for proficiency testing

Most RNA-based genomic assays are designed to detect differentially expressed genes or profiles. Thus, a proficiency testing program for these assays should involve replicate samples of two biologically different samples with known differences in transcript abundance. For example, if each laboratory processed at least three replicates of sample A (labeled A1, A2, and A3) and at least three replicates of sample B (labeled B1, B2, and B3), the results could be used to evaluate both the within-laboratory repeatability in terms of intensity (e.g., A1 vs. A2) and differential gene expression (e.g., B1/A1 vs. B2/A2). When multiple laboratories are providing data generated using the same RNA samples and the same platform, the comparability of the detected differences in expression between sites (e.g., B/A site 1 vs. B/A site 2) can be assessed. In addition, we recommend that proficiency testing be repeated throughout the year so that multiple data sets from the same laboratory can be

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[12] Thompson KL, Rosenzweig BA, Pine PS, Retief J, Turpaz Y, Afshari CA, Hamadeh HK, Damore MA, Boedigheimer M, Blomme E, Ciurlionis R, Waring JF, Fuscoe JC, Paules R, Tucker CJ, Fare T, Coffey EM, He Y, Collins PJ, Jarnagin K, Fujimoto S, Ganter B, Kiser G, Kaysser-Kranich T, Sina J and Sistare FD (2005) Use of a mixed tissue RNA design for performance assessments on multiple microarray formats. *Nucleic Acids Res* **33**:e187.

[13] Reid LH *et al*. (2006). Proficiency testing program for microarray facilities (in preparation). http://www.expressionanalysis.com/proficiency_test.html.

[14] Shi L, Reid LH *et al* (2006) MicroArray Quality Control (MAQC) Project: A comprehensive survey demonstrates concordant results between gene expression technology platforms. *Nat Biotechnol* **24**(9), 1151-1161.

compared to confirm the consistency of the laboratory's performance over time (e.g., A1 time 1 vs. A1 time 2, and B1/A1 time 1 vs. B1/A1 time 2).

- Laboratory compliance

The Agency encourages microarray facilities to adhere to the good laboratory practices outlined in 21 CFR 58. Laboratories may also wish to obtain CLIA certification if the microarray data have potential clinical or diagnostic applications. All CLIA-compliant assays require repeated data comparisons with other providers to verify the competency of individual laboratories. Participation in a proficiency testing program would fulfill this CLIA requirement.

1.5. Hybridizations for Microarrays

At this point in time, there is a lack of widely accepted QA/QC control metrics for DNA microarray technologies, and there is currently no consensus on how to establish the reliability of the results obtained from a DNA microarray experiment. QA/QC pass/fail filters to eliminate outlier arrays are used by some companies and organizations, and some array manufacturers recommend thresholds for certain platform-specific QC measurements.

It is recommended that pertinent information on reproducibility and accuracy be included in the submission package. Currently, the ERCC (External RNA Controls Consortium)[15] and MAQC groups are developing spike-ins and reference standards, which may be useful in evaluating the quality of a particular microarray experiment in the future. Another recent effort has produced a pair of reference RNAs for use with rat DNA microarrays that allows accuracy, reproducibility, and dynamic range assessments[16] (Thompson et al., 2005). Conceptually, this strategy could be used to produce reference materials for any organism, including human. Until such independent resources are widely available and consensus quality standards are developed and implemented by the microarray community, carefully adhering to the microarray manufacturer's recommended procedures offers the best current practice at this time. Detailed protocols have been prepared by major DNA microarray manufacturers and posted on the MAQC Web site at http://edkb.fda.gov/MAQC/. Because the microarray field is an evolving field, it is important to note that manufacturers occasionally change probe sequences and protocols, reflecting continuing improvements to this technology.

We recommend that the following be clearly outlined in a figure:

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[15] External RNA Controls Consortium. (2005) The External RNA Controls Consortium: a progress report. *Nature Methods* **2:** 731 - 734.

[16] Thompson, K.L., Rosenzweig, B.A., Pine, P.S., Retief, J., Turpaz, Y., Afshari, C.A., Hamadeh, H.K., Damone, M., Blomme, E., Ciurlionis, R., Waring, J., Fuscoe, J.C., Paules, R., Tucker, J., Fare, T., Coffey, E.M., He, Y., Collins, J., Jarnagin, K., Fujimoto, S., Gander, B., Kiser, G., Kaysser-Kranich, T., Sina J., and Sistare, F.D. (2005) Use of a mixed tissue RNA design for performance assessments on multiple microarray formats. *Nucleic Acids Research* **33:** e187 doi:10.1093/nar/g.

- The microarray experimental design details

Include sample processing and labeling (e.g., were samples processed in the same batch or different batches; was the same procedure used for all samples, technical replication, biological replication and other appropriate information).

- How data were generated and analyzed.

Start with how the primary data were obtained (e.g., laser scanner settings), software settings for image acquisition; how the data from individual microarrays were combined, normalization method, data filtering, data analysis, statistical tests, and other appropriate information.

1.6. Fluorescence Reader Settings for Microarrays

DNA microarray technology is a multi-step process in which variability at each step must be reduced to maximize the probability of uncovering biological knowledge. One potential source of variability is errors due to the scanners used to collect the microarray signals. Recent publications have pointed out the importance of optimal reader settings for obtaining high-quality microarray data[17] (e.g., Shi L et al., 2005 and references within). The signal readout system is often thought of as a black box that quantitates the signal from each DNA microarray spot. The measurement of the abundance of RNA species by DNA microarray technology assumes a linear relationship between the signal read-out from the scanner and the dye concentration, which is further assumed to be linearly correlated with transcript abundance in the RNA sample.

Each scanner type and signaling dye combination, however, has its own linear dynamic range, which appears to change with voltage gains. Important recommendations for scanners that will help minimize technical variability and improve consistency of data collection include the following:

1. Calibration of scanners as recommended by the manufacturers
2. Routine use of standardized scanner reference materials for calibrations to allow for characterization of concentration-dependent read-outs
3. Attention to scanner settings (e.g., laser power and voltage gain). Specifically, we recommend that scanner settings be set to maximize the linear dynamic range.
4. Keeping the scanner laser power and voltage settings constant during an experiment. Some scanners are not tunable so that one source of variability is eliminated.

[17] Shi, L., Tong, W., Su, Z., Han, T., Han, J., Puri, R.K., Fang, H., Branham, W.S., Chen, J.J., Xu, Z., Harris, S.C., Hong, H., Xie, Q., Perkins, R.G., and Fuscoe, J.C. (2005). Microarray scanner calibration curves: characteristics and implications. *BMC Bioinformatics* **6 (Suppl 2)**:S11.

5. If the dye-intensity to signal output relationship is defined, possible corrections when signals fall outside of the linear dynamic range, thus reducing variability in the very high or very low signal range
6. Submission of scanner setting and calibration information as part of the submission package

1.7. Differentially Expressed Genes

Specific sets of genes derived from microarray experiments can be proposed as genomic biomarkers for a specific endpoint. Such specific gene sets can be reproduced upon review if the analysis protocol and resulting differentially expressed genes are identical to those reported by the sponsor. The sponsor should include in the submission a clear description of the steps, parameters, and algorithms leading to the differentially expressed gene list in the genomic submission.

Different analysis protocols may yield dissimilar differentially expressed genes, and these cannot be justified solely through a biological interpretation if they are to be proposed as genomic biomarkers. To the extent that these genomic biomarker sets become part of a decision-making process in drug development or therapeutic applications, we recommend that transfer of genomic biomarker sets from microarrays to other platforms (such as quantitative RT-PCR) be attempted only after the sponsor concludes that these are sensitive, specific, and reproducible.

Sources of variability in microarray data leading to the step in which the differentially expressed gene list is to be determined may be minimized by following the recommendations in this document. As the step is reached in which differentially expressed genes are to be determined, a number of factors need to be considered that have confounding effects on the generation of these:

- the application of platform-specific flags
- rejection criteria for low-intensity transcripts
- rejection criteria for outlier hybridizations
- platform-specific normalization protocols
- analysis protocol for selection of differentially expressed genes

There is no consensus at this time regarding the choices needed for each of these factors. The sponsor should exercise care in how parameters and protocols are chosen for each of these factors and should consult current literature[18, 19, 20, 21, 22, 23] regarding efforts to reach a consensus on these.

[18] Simon R. Development and evaluation of therapeutically relevant predictive classifiers using gene expression profiling. (2006) *J Natl Cancer Inst.* **98**(17):1169-71.

[19] Simon R. (2006) A checklist for evaluating reports of expression profiling for treatment selection. Clin *Adv Hematol Oncol.* **4**(3):219-24.

In principle, several analysis protocols can be used to determine differentially expressed gene lists for a sufficiently large number of technical and biological replicates. In practice, constraints on the number of technical and biological replicates are likely to be the norm in genomic submissions. For example, technical replicates are constrained by the minimum amount of RNA needed to hybridize each biological sample. Both clinical as well as preclinical samples may have major constraints in the total amount of RNA available from each biological sample. Biological replicates are constrained by the total number of patients or animals to be included in a study. We recommend that these constraints be considered in the selection of analysis protocols for the determination of differentially expressed genes.

1.8. Biological Interpretation of Lists of Differentially Expressed Genes

Once the list of dysregulated genes has been generated via a variety of statistical and analytical tools, the next step in the process is to interpret the biological meaning of gene expression changes and determine whether biological pathways may be of functional relevance to the mechanism of drug action, or maybe correlated to safety and/or efficacy.

A number of questions should be addressed at this point. These questions may include:

- Are genes from a particular pathway or set of pathways significantly overrepresented in the list?
- How many pathways are affected?
- Can the mechanism of action be inferred from the functions of the pathways altered or from the pattern of expression across the genes within these pathways?
- What is the tissue specificity of the pathways and the gene function in relation to biological processes?
- What are the magnitude and/or pattern of the alteration in a particular pathway in relation to treatments with other compounds (related or unrelated) with known pharmacological or toxicological properties?

At present, no single tool can be used to find answers to all these questions, but a combination of tools can be used to address a particular question of interest as thoroughly as currently possible.

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[20] Dobbin KK, Simon RM. (2006) Sample size planning for developing classifiers using high dimensional DNA microarray data. *Biostatistics.* [Epub ahead of print].

[21] Varma S, Simon R. (2006) Bias in error estimation when using cross-validation for model selection. *BMC Bioinformatics*. **7**:91.

[22] Guo L, Lobenhofer EK, Wang C, Shippy R, Harris SC, Zhang L, Mei N, Chen T, Herman D, Goodsaid FM, Hurban P, Phillips KL, Xu J, Deng X, Sun YA, Tong W, Dragan YP, Shi L. (2006) Rat toxicogenomic study reveals analytical consistency across microarray platforms. *Nat Biotechnol.* **24**(9):1162-1169.

[23] Canales RD, Luo Y, Willey JC, Austermiller B, Barbacioru CC, Boysen C, Hunkapiller K, Jensen RV, Knight CR, Lee KY, Ma Y, Maqsodi B, Papallo A, Peters EH, Poulter K, Ruppel PL, Samaha RR, Shi L, Yang W, Zhang L, Goodsaid FM. (2006) Evaluation of DNA microarray results with quantitative gene expression platforms. *Nat Biotechnol.* **24**(9):1115-22.

To this end, a variety of analytical platforms are available, either free on the Web or via purchase of a commercially available product.

An overlap of the biological interpretations obtained with two or more different databases can facilitate a consensus on what the interpretation should be. However, this is not always the case. Consensus can be hindered by many factors including, but not limited to, absence of information on the compound of interest in the reference databases or a lack of annotation for particular pathways of interest. For example, subsets of genes may be placed in specific pathways in one system, but they may not be represented in the same pathways in another pathway analysis tool, or genes may not have been evaluated in a particular platform. In pathway analysis databases, the information may differ depending on which content is extracted from the literature and how that extraction is performed (whether automated or by manual curators). In addition, a critical distinction is whether all information is extracted, or if only the information supported by direct experimental evidence included in the publication is extracted. We recommend heavy reliance on the literature and on reference databases to extract functional information on specific gene lists and generate hypotheses on the biological significance of the relevant set of genes.

We also recommend that the biological significance of gene sets as proposed by a sponsor be accompanied by a standard set of information that will enable recapitulation of the analysis and assessment of the validity of the interpretation by regulatory reviewers. Such information should include, but not be limited to:

- Type of database used for annotation, including vendor name
- Methods and approaches (cut-off, statistical tests) used to identify over-represented pathways within the database
- References used to justify any user-defined annotation
- A summary by the sponsor of the interpretation of the pathway annotation results

2. GENOTYPING

2.1. Genotyping Methods

Genetic differences among individuals occur in a variety of forms, from gross chromosomal alterations to single base-pair changes. The type of genetic variation most often important in pharmacogenetics occurs at the level of individual genes (e.g., drug metabolizing enzymes) on a scale ranging from single base-pair changes to entire gene duplications or deletions. Examining genomic DNA is usually the most reliable and practical method for characterizing genetic variation, although methods based on protein or mRNA expression levels can be preferable in some situations, such as when determining treatment-sensitivity of cancer or viral infection. Many methods are currently available for characterizing DNA variations, and new methods are rapidly being developed.

2.2 DNA Isolation, Handling and Characterization

Whole blood is commonly used for the extraction of genomic DNA in clinical research settings. Blood collection tubes generally use anticoagulants such as EDTA, CPD, ACD, Citrate or Heparin. DNA in a blood sample can undergo rapid degradation in less than 1 week at room temperature. Storage conditions recommended by manufacturers of blood collection tubes should be followed for blood storage.

Carryover of contaminants such as salts, phenol, ethanol, heme (in blood DNA isolation), and detergents from conventional purification procedures can inhibit performance of DNA in downstream applications. In addition, contamination with heparin (an anticoagulant commonly used in blood collection tubes) impairs amplification by PCR.[24] [25] Contamination should be strictly avoided.

Although DNA is a relatively stable molecule, it should be stored carefully. Introduction of enzymatically active nucleases to DNA solutions should be avoided, as these enzymes will degrade DNA. DNA is subject to acid hydrolysis when stored in water, and should therefore be stored at a slightly alkaline pH. Degradation of DNA can have a major effect on any results obtained, generating errors that are both quantitative and qualitative. For example, a reduction in DNA size may lead to the failure of downstream applications such as PCR-based applications and hybridization. Long-term storage of DNA in a slightly alkaline pH (e.g., in Tris-EDTA buffer) at -20^0 C or at -80^0 C is recommended. To reduce repeated freeze-thaws, it is recommended that the sample be frozen in aliquots.

2.3. Genotyping Report

We recommend that the following information be included in the genotyping report, regardless of the genomics submission type (see the *Pharamcogenomic Data Submissions* guidance for regulatory requirements):

- Description of assay platform or methodology
- Samples studied, including demographics and sample size justification for genotype/clinical phenotype correlation and adequate coverage for ethnic/racial groups
- Alleles measured and correlation with metabolic status designation
  - For metabolizing enzymes, how EM (extensive metabolizer), PM (poor metabolizer), IM (intermediate metabolizer), or UM (ultra rapid metabolizer) are determined.
  - Sample test report.
  - For new genes, correlation between gene variant and encoded protein activity.
- Whether the assay was performed in a CLIA certified lab or research lab

[24] Smythe et al., BMC Infectious Diseases, 2002, 2:13.

[25] Yokota et al., Journal of Clinical Laboratory Analysis, 1999, 13: 133 – 140.

3. GENOMIC DATA IN CLINICAL STUDY REPORTS

There are many possible sources of data for genomic data submissions. Genomic data from clinical studies may result from microarray expression profiling experiments, genotyping or single-nucleotide polymorphism (SNP) experiments, or from other evolving analytical methodologies pertaining to drug dosing or metabolism, safety assessments, or efficacy evaluations. Genomic data may also be reported from studies where other data are also reported, such as with efficacy or safety data from clinical or nonclinical studies. However, these data can be reviewed only if the content of the clinical data report included in the submission contains ***sufficient detail*** regarding the sample selection,

The following describes FDA's current thinking about what data should be submitted with genomics data in a submission to the Agency (including a voluntary submission). Regulatory applications for these data are described in detail in FDA's *Pharmacogenomic Data Submissions* guidance in the context of different algorithms for the submission of pharmacogenomic data consistent with FDA requirements for INDs, NDAs, and BLAs, as well as for Voluntary Genomic Data Submissions (VGDS). Throughout the following discussion, we suggest that you refer to the *Pharmacogenomic Data Submissions* guidance for in-depth background on this discussion.

In all genomic submissions, a full clinical study report is very helpful to Agency reviewers. The report should provide a clear explanation of how the critical design features of the study were chosen as well as enough information on the plan, methods, and conduct of the study to eliminate ambiguity in how the study was carried out. The report with its appendices should also provide individual patient data relevant to pharmacogenomics, including demographic and baseline data, and details of analytical methods such as validation reports to allow replication of the critical analyses. It is also particularly important that all analyses, tables, and figures carry clear identification of the set of patients from which they were generated.

To improve the usefulness of the submission, we recommend that the content of the clinical section describing a genomic experiment contain the following information:

- Title page
- Table of contents
- Synopses and summary of findings
- Background and scientific rationale
- Primary and secondary study objectives
- Study design, sample collection and pharmacogenomic methods
- Clinical study protocol., including minimally[26]:
  - inclusion and exclusion criteria
  - demographic data
  - listing of individual experimental measurements by patient, including pharmacokinetic/pharmacodynamic datasets and lab results; and explanation of missing data

[26] See also the format and content specified in ICH guidance *E3 Structure and Content of Clinical Study Reports.*

- disposition of patients
- protocol deviations
- individual adverse events or laboratory abnormalities
- pharmacogenomic and other biomarker datasets as necessary
- correlation between clinical and pharmacogenomic data
- discussion and conclusions

– When available and appropriate, references and any supplementary materials

The specific sequence and grouping of topics may change if alternatives are more logical for a particular study. Some information in the appendices of this report (such as the data handling report and the case report) are required by regulation and must be submitted to the Agency. The *Pharmacogenomics Data Submissions* guidance and other Agency regulations and guidance contain detailed discussions on specific regulatory requirements.

The preferred submission standard for clinical data is the Clinical Data Interchanges Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) standard. Please see the FDA Data Standards Council Web site at: http://www.fda.gov/oc/datacouncil/ for more information on the standard.[27]

4. GENOMIC DATA FROM NONCLINICAL TOXICOLOGY STUDIES

Genomic data can be collected in nonclinical studies, such as toxicogenomic studies. This section describes how to submit nonclinical toxicology data with a genomic data submission. How the data should be submitted depends on the purpose of the submission. Three general types of submissions can be identified:

- The first might have the objective of expanding the selection process criteria (i.e., screening to aid in the selection of a lead compound for clinical development or to eliminate compounds with certain characteristics).
- The second might present the characterization of a particular compound.
- The third might present a general scientific discussion that might not be related to the development of a compound and/or compound class.

[27] The SDTM can be obtained from the CDISC Web site at: http://www.cdisc.org/models/sds/v3.1/index.html .

SDTM Implementation Guides:

- *The Study Data Tabulation Model Implementation Guide (SDTM-IG) for clinical study data* can be obtained from the CDISC web site at: http://www.cdisc.org/models/sds/v3.1/index.html
- *The Study Data Specification for submitting SDTM datasets to CDER* can be obtained at http://www.fda.gov/cder/regulatory/ersr/Studydata-v1.1.pdf

PK/PD data submission should be in SAS.XPT-compatible format.

4.1. Expanding the selection process criteria.

When a submission is intended to expand the selection process criteria and precede the development of a compound (i.e., screening for lead compounds or to eliminate certain characteristics), we recommend the inclusion of the following information.

1. General narrative about the objective of the submitted application, brief narrative about the compound(s), intended use, and mechanism of action
2. Objective of the submitted study with its experimental design (treatment, duration, replicates, drug formulation, route of administration, rationale for dose selection). As applicable, information about species, strain, sex, genetic background, age, weights, developmental stage, organ/tissue where sample originated, cell type can be included. We recommend that a brief description of sample handling and preparation methodology also be included.
3. Toxicology parameters including clinical pathology (serum chemistry and hematology) and histopathology data consistent with STP guidelines (Toxicologic Pathology, 32, 126-131 (2004)), preferably in an electronic format). When applicable, the correlation between pathology findings and genetic variation or gene or protein expression should be explained.
4. Correlations of individual animal data to genetic variation or gene or protein expression should be explained.
5. Pharmacokinetic parameters and ADME properties of the compound should be provided if known. When applicable, correlation between pharmacokinetic findings and genetic variation or gene or protein expression should be highlighted.
6. Reference should be made to scientific and analytical methods for genetic variation or gene or protein expression, including genotyping or expression profiling methods, statistical methods, and software packages used.

4.2. Characterization of a particular compound.

If the intent of a submission is to characterize a particular compound, it is generally recommended that the toxicology portion of the submission be reported in a similar format to a toxicology report. These reports follow the good review practices template (Section 4.1 m (1 to 6)). If the template is not used, a copy of the study protocol should accompany the line listings and generally include clinical signs, mortality, body weight, food consumption, hematology, clinical chemistry, urinalysis, gross pathology, organ weights, histopathology, and pharmaco/toxicokinetics (as available) with a full tabulation of data suitable for detailed review. These data contain line listings of the individual data points, including laboratory data points, for each animal along with summary tabulations of data points. A copy of the study protocol is expected to accompany the line listings.

4.3. General scientific discussion.

When a submission contains data to support a general scientific discussion that is not necessarily related to the development of a compound and/or compound class, the minimal amount of

nonclinical data to be submitted should be similar to the previously described scenarios. However, it is up to the sponsor to provide adequate information to clarify and support the scientific issues discussed. The data submitted will probably not be detailed, but we recommend that it be tabulated in a form that will be concise and adequately descriptive for the specific purpose of the submission.

For any type of genomic data submission, we encourage you to submit the data electronically in a tab-delimited file conforming to the Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model (SDTM) standard or the Standard for Exchange of Nonclinical Data (SEND) SDTM format per the CDISC guidelines (http://www.cdisc.org/).[28]

[28] More information can be found at FDA Data Standards Council Web site, http://www.fda.gov/oc/datacouncil/.The *Standard for Exchange of Nonclinical Data (SEND) Implementation Guide for Animal Toxicology Studies* can be obtained from the CDISC Web site at: http://www.cdisc.org/models/send/v2.3/SENDV2.3ImplementationGuide.pdf.